

## RESEARCH ARTICLE

### SERICULTURE IN CUBA: SCIENTIFIC ADVANCES AND CHALLENGES

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#### ABSTRACT

Cuba's National Sericulture Project was started in 2011, in order to develop products from the silkworm cocoon for the biopharmaceutical, biotechnological and cosmetic industries. The Project has developed its scientific foundations in the course of these years, as well as the scaling of silkworm rearing in three stages. This paper shows results and implemented methodologies in the period 2011-2021. The results of the efficiency rate for the Integrated System *Morus alba-Bombyx mori* with an agroecological approach, are also presented. The main products derived from the silkworm cocoons so far, have been silk thread, sericin hydrolyzed and fibroin nanoparticles. The main challenges of the Project are focused on the incorporation, evaluation and development of new breeds of *Bombyx mori* L.; egg production; the expansion to production scale through an integrated rearing management, all these based on a circular economy, as well as the attainment and development of new products from the silkworm cocoon.

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## INTRODUCTION

The introduction of Sericulture in Cuba dates back to the XIX century (1824-1945). In that stage, Sericulture projects were developed in the central part of the island (Santa Clara) and Havana (Santiago de las Vegas). However, late in the 40's of the XX century, the activity was abandoned due to technical problems of the productive chain, the competence of artificial silk and the lack of scientific foundations to face such adversities (Tirrelli, 1939). In 2001, a project for rearing silkworm (*Bombyx mori* L.), was started in order to encourage craftsmanship abilities in disabled persons to process cocoons; thus, starting a new period of Sericulture in Cuba that included the introduction of *Bombyx mori* L. eggs into the country considered as exotic organisms and since then, they have been subjected to regulations by the National Cuban Center for Biological Safety. Late in 2011, a National Sericulture Project was started in Havana in order to develop a sustainable sericulture chain value in large-scale, to promote the use of the silk thread and different byproducts of sericulture for the textile, pharmaceutical, cosmetic and biotechnological industries, taking advantage of the strengths of different scientific and productive institutions with a large path and prestige in the country.

This challenge implied the development of scientific and methodological foundations of Sericulture in Cuba with 9 rearing per year including breeds that were able to adapt to Cuba's environmental conditions (Pérez-Hernández, *et al.*, 2017). This research shows a review of the main scientific results attained since 2011 so far, regarding large-scale rearing of *Bombyx mori* as well as the challenges of the Cuba's Sericulture Project.

#### General management and rearing scaling of silkworm:

From 2012 to 2016, the behavior of different breeds of *Bombyx mori* L from different origins was studied: Brazil, Bulgaria, China, Colombia, Korea, Spain, India, Italy and Thailand. The main productive and quality indicators were compared (Cifuentes and Sohn, 1998 and Pescio, 2009), in so doing, a sample of 50 cocoons was used, the rest of the indicators were evaluated for the equivalent of 25 thousand cocoons contained in a box according to international commercial standards. The best results were attained with eggs from Thailand breeds (Chul Thai 6 and Chul Thai 7) and that from China. These breeds had not been evaluated before in the country (Table 1). The analysis of such indicators between two breeds from Thailand and one breed from China showed significant higher values for Thailand breeds compared to the Chinese one for a  $p < 0.05$ . Likewise, the comparison between the Thailand breeds showed that indicators like the length and width of the cocoon, production per box and percentage of

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gross silk, were significantly higher for the Chul-Thai 6 breed in relation to Chul-Thai 7. Indicator values are shown in table 1, according to the features reported under the conditions of each country (Qin *et al.*, 2020 and Minarningsih, *et al.*; 2021). Cocoon production per box was 30-37 kg and production of gross silk ranged from 20 to 25%, both parameters were in tune with the reports found in the literature (Qin, *et al.*, 2020 and Minarningsih, *et al.*; 2021).

Since 2016, the evaluation of new breeds has continued with breeds from China and India in order to continue with the search for breeds adapted to the conditions of humid tropical areas and for the production of silkworm eggs that will be published further on.

**Productive scaling of silkworm rearing:** The scaling was done in three stages, the first one included 3 boxes per rearing, the second one 10 boxes per rearing and a third one 30 boxes per rearing. The first stage validated the biological cycle at a higher scale of the selected breeds under Cuba's environmental conditions, showing the possibility of shortening the duration of the larval stage from 25-28 days (Cifuentes and Sohn, 1998; Pescio, 2009; Gurjar, *et al.*, 2018) to 21-23 days, which led to 9 rearing per year. This result allows for saving mulberry since its consumption by the silkworm is reduced in two days, the incidence of diseases that generally appear in the last ages is also reduced. All this shows the adequate technical and environmental conditions established. Massiveness in rearing brought about the presence of diseases and it was necessary to perform effective disinfection cycles to reduce the incidence of pathogens in the rearing rooms and prevent the establishment of diseases endemically, and make some research on the main diseases affecting the rearing of *Bombyx mori* L. under our conditions. The most common diseases causing major damages are those caused by the nuclear polyedrosis virus *Bombyx mori* (BmNPV), (Grassery) and by bacteria (Flachery) that causes diarrhea and body flabbiness to the silkworm (Cifuentes and Sohn, 1998; Sharma, *et al.*, 2020). *Serratia marcescens*, *Streptococcus* sp, *Enterococcus* sp, *Staphylococcus* sp have been identified, among others. The results from virus studies: biological, pathogenic and molecular characterization, allowed to determine that symptoms associated to Grassery in massive production systems of *Bombyx mori*, had, as essential cause, the presence of the nuclear polyedrosis (BmNPV), which was the first report of this pathogen in Cuba's sericulture (Martínez-Zubiaur, *et al.*, 2016).

The results of bacteriological analyses made to basic morphological, biochemical and physiological tests allowed to identify isolates like *Enterobacter cloacae*, *Serratia marcescens* and *Acinetobacter baumannii*, which were reported in the literature as pathogens of insects (Díaz-Sánchez, *et al.*, 2014; Gandotra *et al.*, 2018 and Yin *et al.*, 2018). This study made possible to establish programs to prevent and control these pathogens in order to avoid increased losses in production. A working methodology was prepared for the administration of medicines and disinfectants to avoid and control diseases. This methodology was evaluated in further rearing and incorporated to the Cuba's integrated rearing approach, thus allowing to reduce mortality at levels of 3,2-7,4 % in rearing of 30 boxes. This reduction was also influenced by the prevailing climatic conditions at rearing, the breed and nutrition.

**Silkworm egg production:** The Sericulture Project, since its beginning and until 2016, systematically imported silkworm eggs from Thailand and China. From 2017 to 2023, sporadic imports of silkworm eggs were made, but based on research and scientific results, it was possible to produce eggs domestically and partially replace imports gradually. An egg production methodology was developed, similar to the one described by the literature (Kawakami, 2002 and Dandin and Giridhar, 2010), such procedure has been validated in the rearing from 2017 on, with results within the established quality parameters (hatching percentage, mortality and cocoon weight). Due to the novelty of this scientific result and its economic importance for the sustainability of the Cuba's Sericulture Project, work is being done to create a germplasm collection with silkworm breeds and thus set up a breeding program with the international collaboration of countries like Bulgaria, the People's Republic of China and India.

**Optimization of the incubation:** During the third stage of the scaling, the eggs incubation process was optimized by achieving temperature, light and humidity controls. An eggs incubation methodology was developed and once it was implemented, hatching increased to 95-97 %, in one day only and with a higher uniformity, similar to international standards (Dandin and Giridhar, 2010; Matsuoka and Sakamoto, 2018).

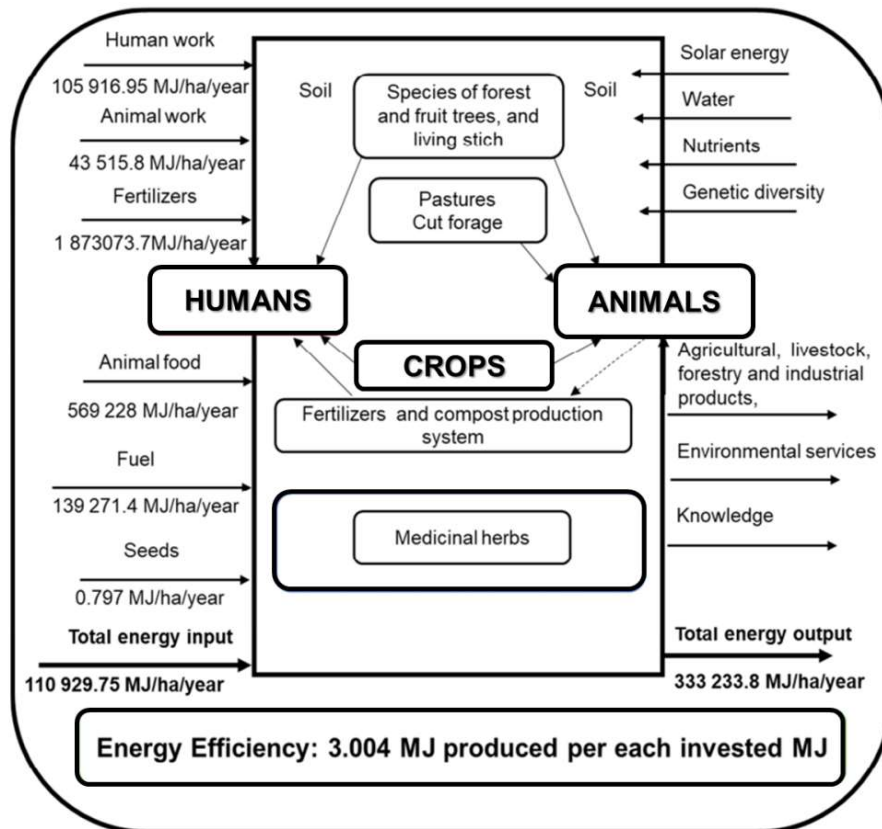
**Drying and conservation processes of silkworm cocoons under production scale:** A drying methodology allowing the storage of cocoons up to two years was developed, which coincides with the time mentioned in the literature (Cifuentes and Sohn, 1998; Pescio, 2009). In our case, due to the climatic conditions of the country it is necessary to store them in cool temperatures of 25 °C and a relative humidity of 85 %.

**Energy Balance in the *Morus alba-Bombyx mori* Integrated System in areas of the National Sericulture Project:** Based on international experiences to find a more efficient sericulture model (Jiping and Ziran, 2015), energy efficiency analyses of the Project in Cuba have been made since 2017.

From an agro-ecological approach, the evaluated system in 2020 can be classified as a bio diverse one with the combination of plants, animals and wildlife in forests and mangroves. This feature has had a positive expression regarding a study made in 2017 (Pérez-Hernández, *et al.*, 2017), related to increased biodiversity of plots and the elimination of intensive single crop of mulberry and the incorporation of sheep, pigs and goats. Figure 1 shows a diagram of energy model attained for the *Morus alba-Bombyx mori* Integrated System. The result of the energy efficiency for the Integrated System of *Morus alba-Bombyx mori* is 3.004 MJ produced by each invested MJ. If the Sustainable Energy Model for food production and energy of Funes, F., (2009) (Energy Efficiency Rate: 6.000) (Funes-Monzote, 2009) is compared to the one attained, it has an intermediate energy efficiency.

**Table 1. Average values of silkworm rearing of two breeds from Thailand and one from China**

Cycleduration (days)	Hatching (%)	Mulberry consumption	Cocoon individual weight	Weight Percentage of the coat	Cocoon length	Cocoon width	Cocoon production per box	Gross silk
		(kg/box)	(g)	(%)	(mm)	(mm)	(kg/box)	(%)
<b>Origin: Thailand breed: Chul-Thai 6</b>								
21	95	728,4	1,91 <sup>a</sup>	22 <sup>b</sup>	34,7 <sup>a</sup>	20,8 <sup>a</sup>	36,1 <sup>a</sup>	25 <sup>a</sup>
			<b>Origin: Thailand breed: Chul-Thai 7</b>					
			1,79 <sup>b</sup>	23 <sup>a</sup>	31,4 <sup>b</sup>	18,6 <sup>b</sup>	35,1 <sup>b</sup>	24,2 <sup>b</sup>
<b>Origin: Chinese breed: Lianguang 2</b>								
21	95	810,5	1,41 <sup>c</sup>	20 <sup>c</sup>	23,4 <sup>c</sup>	14,4 <sup>c</sup>	29,2 <sup>c</sup>	19,7 <sup>c</sup>



**Table 2. Results of cocoon classification of three breeds attained in Cuba according to the quality standards of the People’s Republic of China**

Breed	Quality indicator	Unit of measure	Value of the standard	Test result	Classification
Chul Thai 1	Cleanness	points	≥96.5<97.5	99.0	6 A
	Sharpness	points	≥92.00<94.00	93.0	6 A
	Gross silkyield	%	-	46.62	47
	Length of the continuous thread	M	≥300<1000	913.8	91
	Spinning per 10 000 m	time	≤3	0.4	I
<b>TOTAL RESULT</b>		6 A 4791			
Chul Thai 6	Cleanness	points	≥96.5<97.5	97	4 A
	Sharpness	points	≥92.00<94.00	93	4 A
	Gross silkyield	%	-	37.92	38
	Length of the continuous thread	M	≥300<1000	536.4	53
	Spinning per 10 000 m	tiempo	≤3	2.0	I
<b>TOTAL RESULT</b>		4 A 3853			
FC 2	Cleanness	points	≥96.5<97.5	98.0	6 A
	Sharpness	points	≥92.00<94.00	94.50	5 A
	Gross silkyield	%	-	39.36	39
	Length of the continuous thread	M	≥300<1000	494.3	49
	Spinning per 10 000 m	time	≤3	0.8	I
<b>TOTAL RESULT</b>		5 A 3949			

**Table 3. Quality requirements of the Cuban's sericin hydrolyzed compared with the one sold in the international market**

Quality requirements	Sericin hydrolyzed from silk sold at the international market	Sericin hydrolyzed
Organoleptic features	Clear and pale yellow liquid of characteristic odor	Clear and pale yellow liquid of characteristic odor
Protein concentration	1,2-2 mg/mL	4-8 mg/mL
Relative density at 20 °C	1,099-1,109 g/L	0,985-1,109 g/L
pH	4-5	4-5,5
Microbial load UFC in 0.1 g o ml <100.	NO	NO
Presence of thread-like fungi and of <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Pseudomona aeruginosa</i> , <i>Salmonella typhi</i> .	Absence	Absence

Note UFC Colony Forming Units.

Hence, it is a challenge for the sustainability of Sericulture in Cuba, to achieve an efficient use of available energy sources in the agricultural and livestock *Morus alba-Bombyx mori* system, in order to reach an adequate balance between energy, productive, economic efficiency and people's wellbeing, through innovations, introduction of new scientific results leading to mitigate climate change effects with a value chain based on a circular economy that makes it more energy efficient (Ruiz et al., 2021).

#### Applications derived from the silkworm cocoon

**Production of silk threads:** In 2016, a joint test was made in China with the Institute of Technology and Sericulture of Jiangsu, in order to evaluate the quality of the silk thread produced by Cuban cocoons, from three breeds, two from Thailand (Chul Thai 1 and Chul Thai 6) and one from India (FC2). The quality standards evaluated comply with the standards GB/T 9176-2006 and GB/T 9111-2006 for cocoons from the specie *Bombyx mori* L. (National Fiber Standartization Technical Committee, 2015; National Fiber Inspection Bureau, 2016).

The results showed (table 2) that Cuban produced cocoons are of good quality to be used in automatic reeling machines which means that cocoons from the Chul Thai-1 breed registered the best results. In 2016, a donation from the People's Republic of China consisting of a pilot plant for the extraction of silk threads was received which eased the production of hanks at semi industrial-scale for the first time in Cuba.

**Production of sericin hydrolyzed:** Altogether with the National Center of Animal and Plant Health, the sericin hydrolyzed is produced. This sericin hydrolyzed complies with the organoleptic features and required parameters for the domestic cosmetic industry when compared to the imported product used before (Table 3). It has made possible for that industry to reduce imports of this raw material since 2017, and research is made on diversifying its use with pharmaceutical purposes with skin generating and protecting effects. Table 3. Quality requirements of the Cuban's sericin hydrolyzed compared with the one sold in the international market.

**Nanoparticles of fibroin:** A methodology to produce nanoparticles of fibroin was developed altogether with the Advance Study Center and Biotechnology and Genetic Engineering Center that will make possible the use of nanoparticles with biomedical and cosmetic purposes, among others.

The development of these new products will permit its sale with the consequent contribution to the economic sustainability of the Project.

## CONCLUSION

The National Sericulture Project, with no less than 10 years of age, shows the feasibility to develop the value chain of sericulture in Cuba, as a sustainable alternative in the agricultural and livestock production. The main identified challenges are: incorporation, evaluation and development of new *Bombyx mori* L., breeds according to the different productive outlets of the project; consolidation of the scientific foundations to produce eggs at a production scale; improve the production-scale capacity through an integrated management of silkworm rearing based on a circular economic model, as well as produce and develop new products from the silkworm cocoon.

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